Detection of Minor Subpopulations of Colorectal Adenocarcinoma Cells Expressing Cancer Stem Cell Markers

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The expression of puitative surface molecular markers of cancer stem cells on human colorectal adenocarcinoma cells was analyzed by flow cytofluorometry. Cell subpopulations expressing markers of epithelial and malignant cells and stem cell markers were identified. Four minor subpopulations with $CD24^+/CD133^+$, $CD44^+/CD133^+$, $CD90^+/CD71^+$, or $CD90^+/CD24^+$ phenotypes meeting this requirement were detected; presumably, those were cancer stem cell subpopulations. These results extend our knowledge on heterogeneity of human colorectal adenocarcinoma cell population and outline new trends of research of cancer stem cell phenotype in these tumors.

Key Words: *colorectal adenocarcinoma; cancer stem cells; surface markers; flow cytofluorometry*

Despite the progress in the diagnosis and treatment of cancer, the incidence of relapses and mortality in Russia and in foreign countries remain high. This is largely explained by insufficient introduction of the results of basic studies of tumor biology, *e.g.* heterogeneity of cancer cells, in the diagnostic and therapeutic technologies. This is true for colorectal cancer, which ranks third by incidence.

According to the modern hierarchic concept of carcinogenesis, tumor tissue includes cancer cells of different differentiation degree: cancer stem cells (CSC), rapidly proliferating poorly differentiated cells, and differentiated cancer cells [7]. The characteristics of CSC are the same as of normal stem cells: they are capable of self-reproduction by symmetrical and asymmetrical division and to differentiation, at least aberrant. Due to this they initiate endless reproduction of abnormal differentiated cells from which tumor tissue largely consists. Hence, it is CSC that initiate malignant growth in the primary focus and in metastases. The CSC population as a prospective target focuses the interest of scientists engaged in development of new therapeutic methods. These cells are the most resistant to the majority of modern methods of antitutmor therapy, which substantiates the need in new methods for their identification and studies.

We tried to detect the phenotypical subpopulations of colorectal cancer cells, presumable CSC.

MATERIALS AND METHODS

Tumor tissue specimens obtained after surgical removal of colorectal adenocarcinoma were plunged in

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PBS (PanEco) with streptomycin (200 μg/ml), penicillin (200 U/ml), and amphotericin B (0.5 μg/ml). Necrotic foci were removed and the preparations were fragmented to pieces of 1-2 mm^3. The fragments were transferred into nutrient DMEM (Gibco) with 10% FCS, collagenase (0.1 mg/ml), streptomycin (100 μg/ml), penicillin (100 U/ml), and amphotericin B (0.5 μg/ml) and incubated at constant stirring at 37°C for 18 h. After incubation, the suspension was filtered through a filter with 70 μ pores (Becton Dickinson) in order to remove cell aggregates.

Immunochemical staining with monoclonal antibodies to human surface antigens CD24, CD34, CD71, CD90, CD44, EpCAM, CD166, CD133, conjugated with fluorochromes allophycocyanine (APC), FITC, or phycoerythrin (PE; Becton Dickinson) was carried out. The fluorescence intensity was analyzed on a FACSAria cytofluorometeric sorter (Becton Dickinson).

RESULTS

Primary analysis of cell material obtained as a result of enzymatic disaggregation of tumor tissue was carried out by measuring the expression of surface markers characteristic of epithelial and malignantly transformed cells, and of cells constituting the tumor stroma. The levels of expression of each studied marker were evaluated by histogram of fluorescence intensity of the dye conjugated with specific monoclonal antibodies.

Our findings indicated that more than 90% cells in specimens of colorectal adenocarcinomas expressed EpCAM epithelial antigen, its high level being characteristic of cancer cells (Fig. 1). In addition, the majority of analyzed cells expressed CD24 mucin epithelial receptor, characteristic of malignant tumor cells in the digestive tract and liver, and CD44 hyaluronic acid transmembrane receptor, participating in tumor cell metastasizing [9]. In addition, CD71 transferrin receptor, often present on actively proliferating cells, was expressed on the cells.

Detection of few cells expressing CD90 is worthy of note (Fig. 1). It is known that high expression of CD90 is detected in connective tissues and on various fibroblast cells [2]. Glycoprotein CD90 (Thy-1), present on endothelial cells and fibroblasts, is directly involved in cancer cell interactions with the stroma and thus regulates tumor growth and metastasizing [5]. Detection of this marker can indicate the presence of few fibroblasts from tumor stroma in analyzed specimens. On the other hand, cell populations contained virtually no endothelial fragments, judging from the absence of CD34 expression.

Obviously, CSC, similarly as normal stem cells, are present as minor subpopulations in populations of better differentiated cells (cancer cells in our study). We detected minor subpopulations by double staining with monoclonal antibodies. Co-expression of markers characteristic of cancer and stem cells was detected by this method. Stem cells markers CD133 and CD166 were analyzed; they are expressed on stem cells in normal tissues and on CSC of some tumors, including actively metastasizing prostatic tumors [6]. CD90 marker was also analyzed; in addition to fibroblasts and other stromal cells, it was found on hemopoietic stem cells and on hepatocellular carcinoma CSC [8].

Analysis of the results of double staining showed several variants of minor colorectal adenocarcinoma subpopulations (Fig. 2). Subpopulations of $CD24^+/CD133^+$ and $CD44^+/CD133^+$ cells were found, their levels being 5.6-10.7 and 15.0-26.9%, respectively. On the other hand, in contrast to other studies [3], our analysis of CD44 co-expression with CD166 found no minor subpopulation of $CD44^+/CD166^+$ cells. As was expected, the majority of analyzed cells (more than 80%) had $CD44^+/EpCAM^+$ phenotype, which precluded regarding this subpopulation as a minor one.

The evaluation of co-expression of CD24 and CD71 markers with CD90 provided interesting results (Fig. 2). The measurements revealed almost equal percentage of $CD24^+/CD90^+$ (7.8%) and $CD71^+/CD90^+$ (7.2%) subpopulations. We previously used CD90 as a tumor stroma fibroblast marker for evaluation of contamination of renal cell carcinoma primary cultures by stromal cells [1]. However, co-expression of CD90 and epithelial marker CD24 in colorectal adenocarcinoma indicated different nature of the detected cell subpopulation. The interest to minor subpopulations expressing CD90 was explained by the fact that non-small cell pulmonary carcinoma CSC, expressing CD90 and exhibiting signs of multiple drug resistance, were described previously [4].

Hence, using surface markers characteristic of cancer or stem cells we detected several subpopulations of tumor cells, putative CSC. Further studies of their clonogenic activity, genetic characteristics, and drug sensitivity are required for understanding the mechanisms of cancer development and for therapy of colorectal adenocarcinoma.

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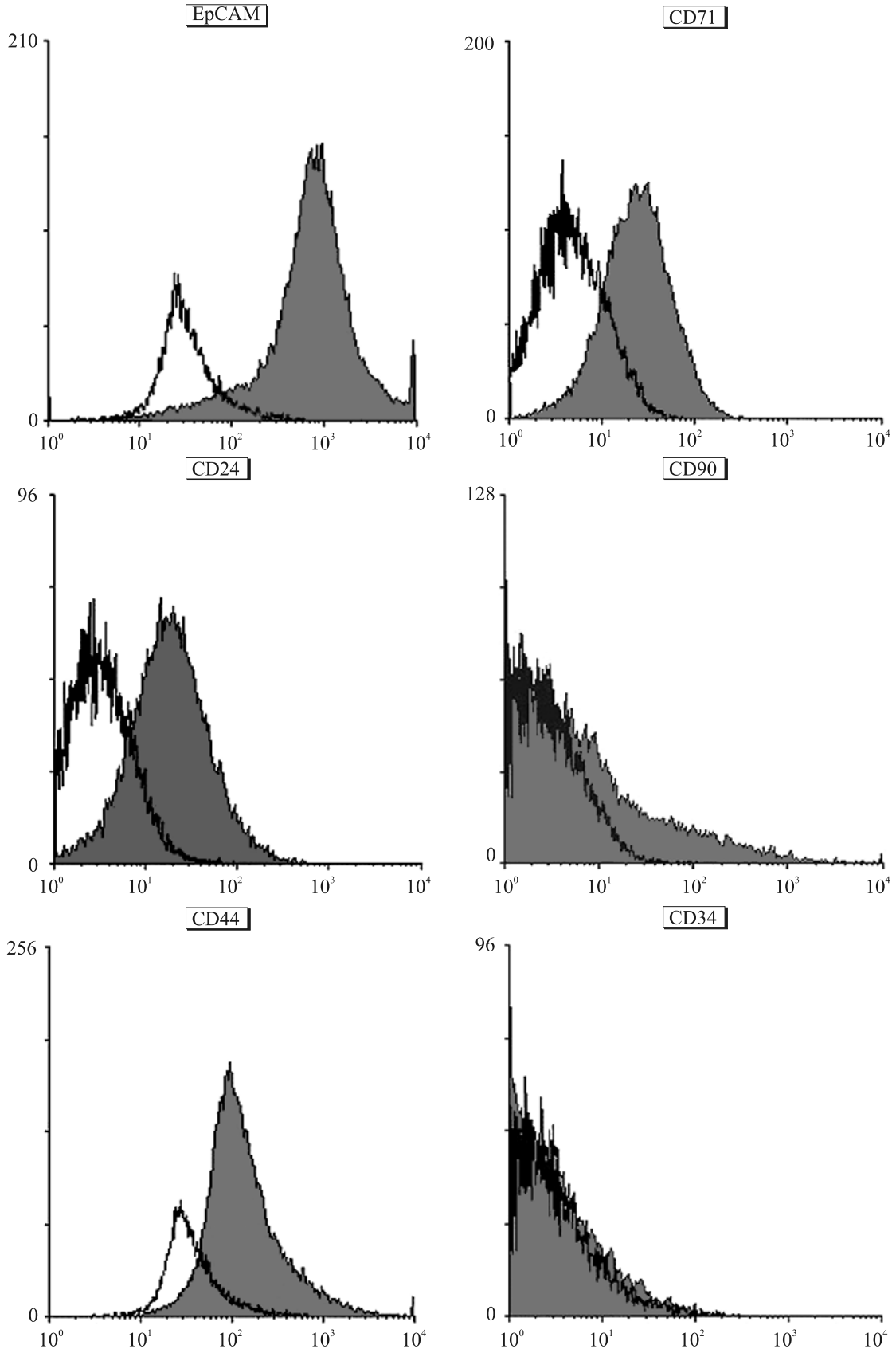

Fig. 1. Expression of surface markers on colorectal adenocarcinoma cells. Abscissa: fluorescence intensity (arb. units); ordinate: number of cells (recorded events). Dark line: isotypical control. The data processed by WinMDI 2.8 software.

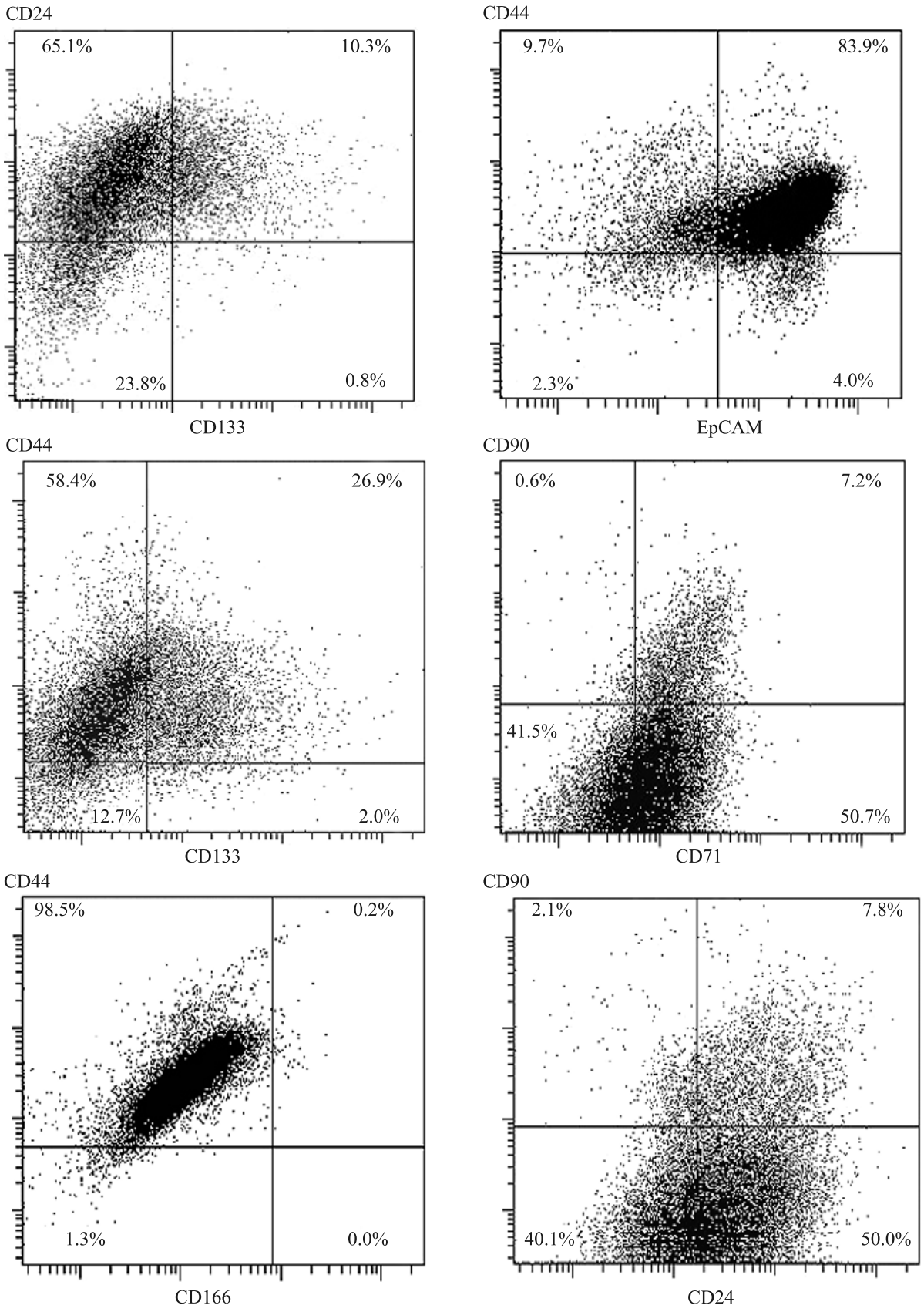

Fig. 2. Minor subpopulations of colorectal adenocarcinoma cells. Abscissa and ordinate: fluorescence intensity (arb. units). Percent content of cells of a certain phenotype *vs*. total cell population is showed in each square.

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